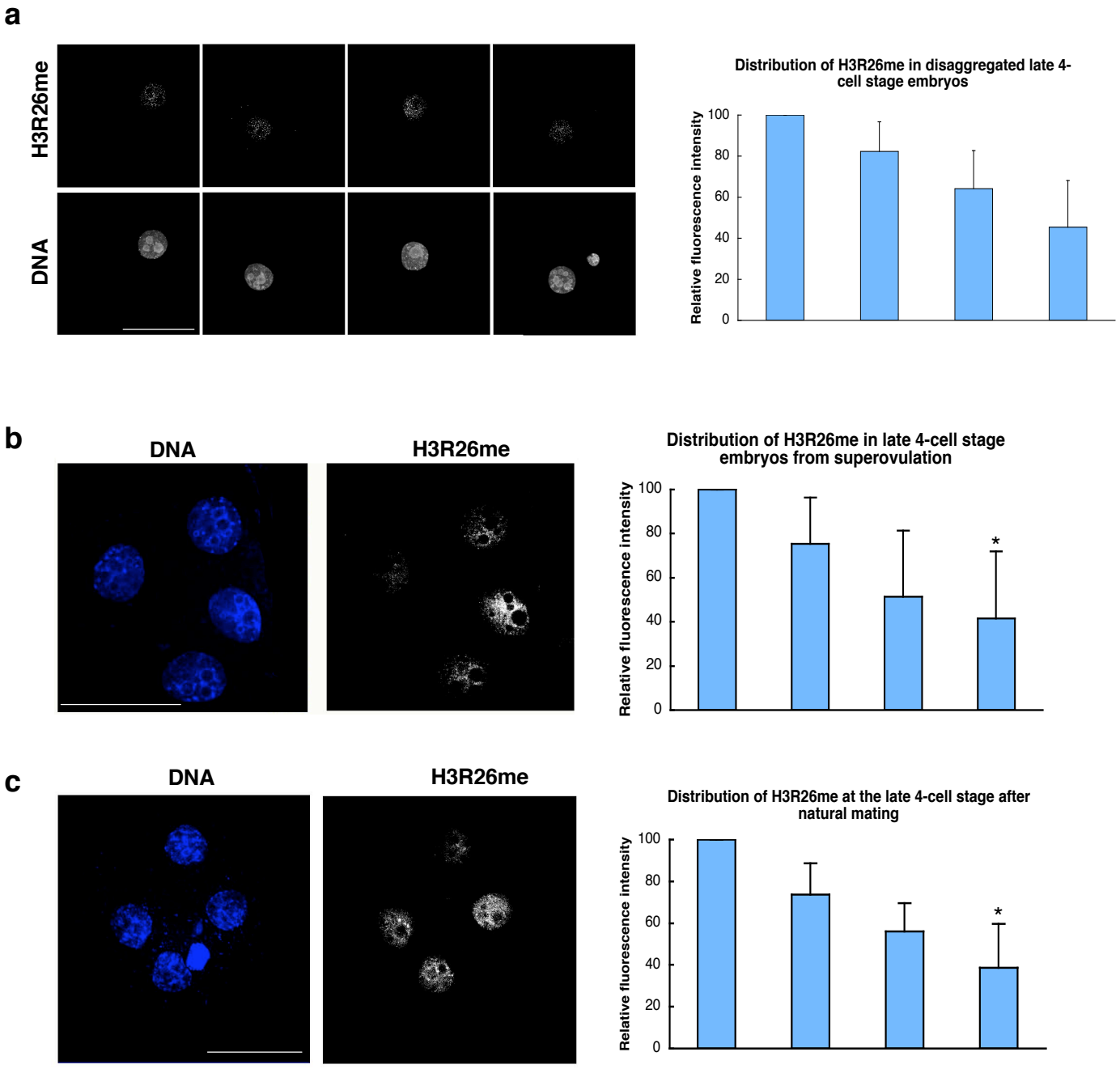

Figure S1. Levels of H3R26me are different in the blastomeres of the 4-cell stage embryo.
(a)Differences in the levels of H3R26me are not an artifact linked to differential scanning caused by embryo shape. Freshly collected late four cell stage embryos were processed for immunostaining with an H3R26me antibody. The cells of individual embryos were then disaggregated and scanned under confocal microscopy separately. Shown are projections of the 4 nuclei of a representative embryo (n=9) . Sections were taken every 0.8 μm. Fluorescence levels were quantified using the Volocity software and normalised against the blastomere showing the highest level which was set at 100%. Decreasing values of fluorescence were calculated, normalised in each embryo and averaged accordingly (n=9). Each bar represents the relative fluorescence level of each of the 4-cell stage blastomeres. Scale bar 10 μm.
(b-c) The variations in the distribution of H3R26me levels at the late 4-cell stage are similar whether they are obtained following hormonally induced superovulation (b; n=18) or derive from natural matings (c; n=14). Embryos were collected and processed as in (a) except that the scanning was done without disaggregating the cells. The levels of fluorescence were quantified in projections including all sections using the Volocity software and plotted as in (a*) (*p*= 0.0001). Shown are projections of representative embryos including all sections, which were taken every 0.8 μm. DNA was stained with TOTO-3. Scale bar 50 μm.

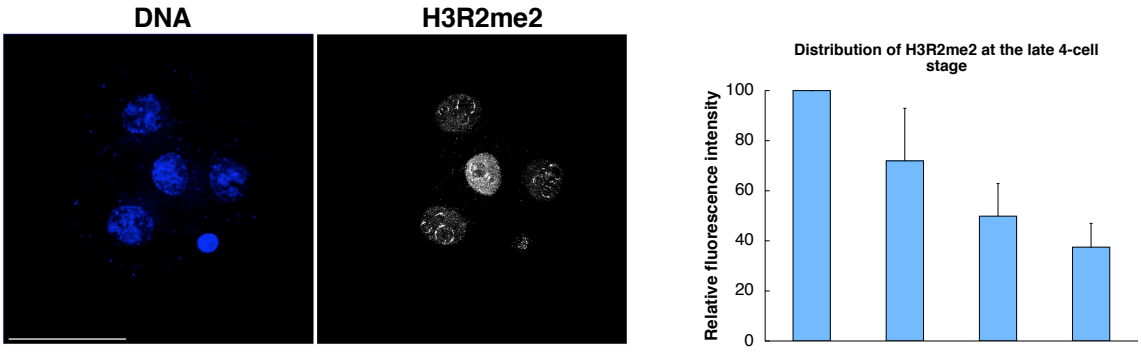

Figure S2. Levels of H3R2me2 are differentially distributed in the blastomeres of the 4-cell stage embryo. Freshly collected late four cell stage embryos were processed for immunostaining with the H3R2me2 antibody and scanned under confocal microscopy. Sections were taken every 0.8 μm. The levels of fluorescence were quantified in projections including all sections using the Volocity software and normalised against the blastomere showing the highest level, which was set at 100%. Decreasing values of fluorescence were calculated, normalised in each embryo and averaged accordingly (n=7). Each bar represents the relative fluorescence level of each of the 4-cell stage blastomeres. Shown is the projection of a representative embryo that include all sections, which were taken every 0.8 μm. DNA was stained with TOTO-3. Scale bar 50 μm.

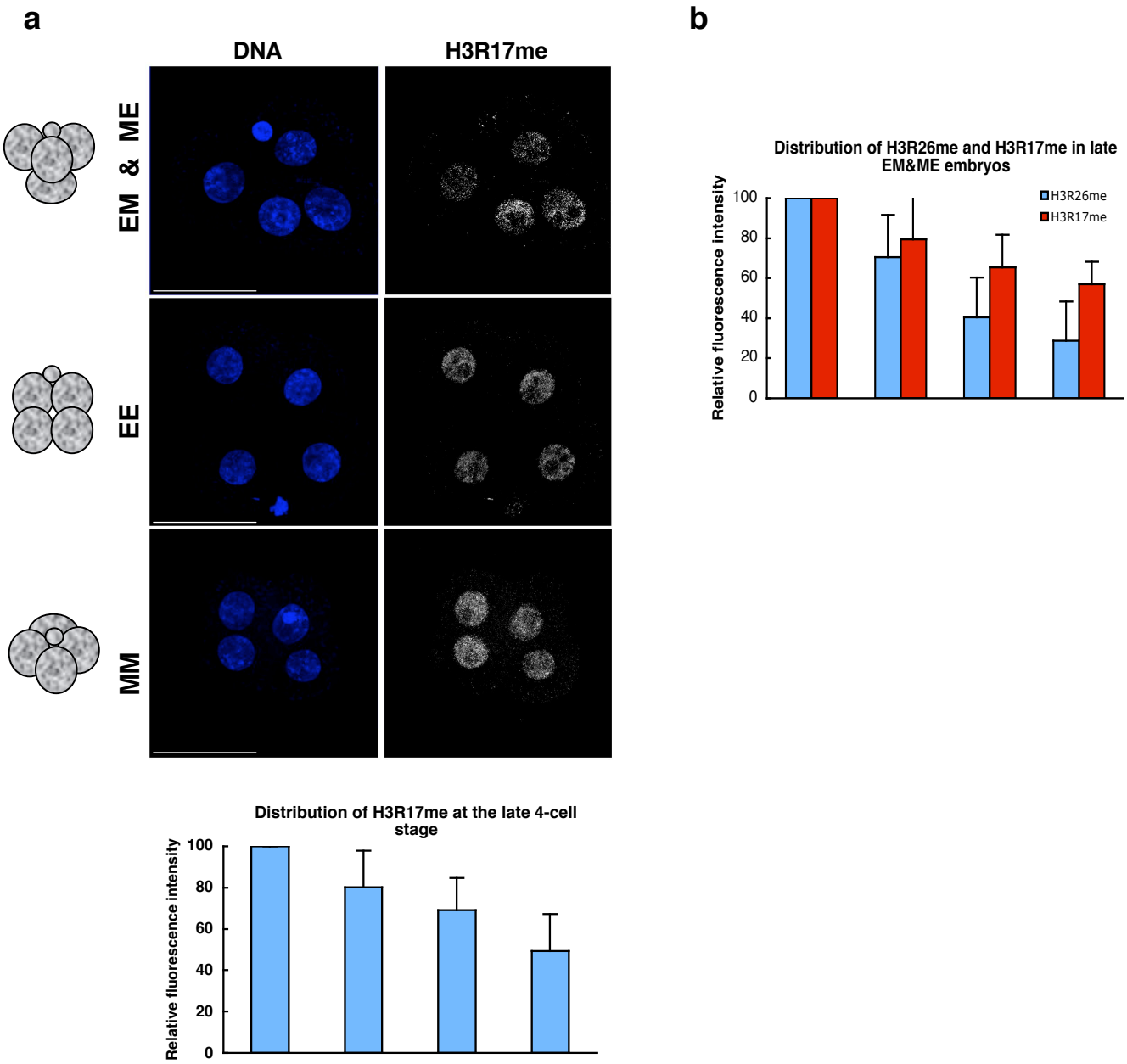

Figure S3. Freshly collected four cell stage embryos were processed for immunostaining with a H3R17me (n=8) antibody and analysed under confocal microscopy. DNA is shown in blue. Shown are projections of representative embryos including all sections, which were taken every 0.8 μm. Embryos were grouped according to their shape in tetrahedral (EM and ME), EE or MM embryos. Scale bar 50 μm. The levels of fluorescence were quantified as in Figure 1. Quantification of the fluorescence levels, which were plotted relative to the blastomere that displays highest fluorescence levels (which was set at 100% for each embryo) is shown. In the graphs, each bar corresponds to the relative levels of fluorescence of single 4-cell stage blastomeres. (b)Comparison of the distribution of H3R26me (blue bars) and H3R17me (red bars) in tetrahedral (EM and ME) 4-cell stage embryos.

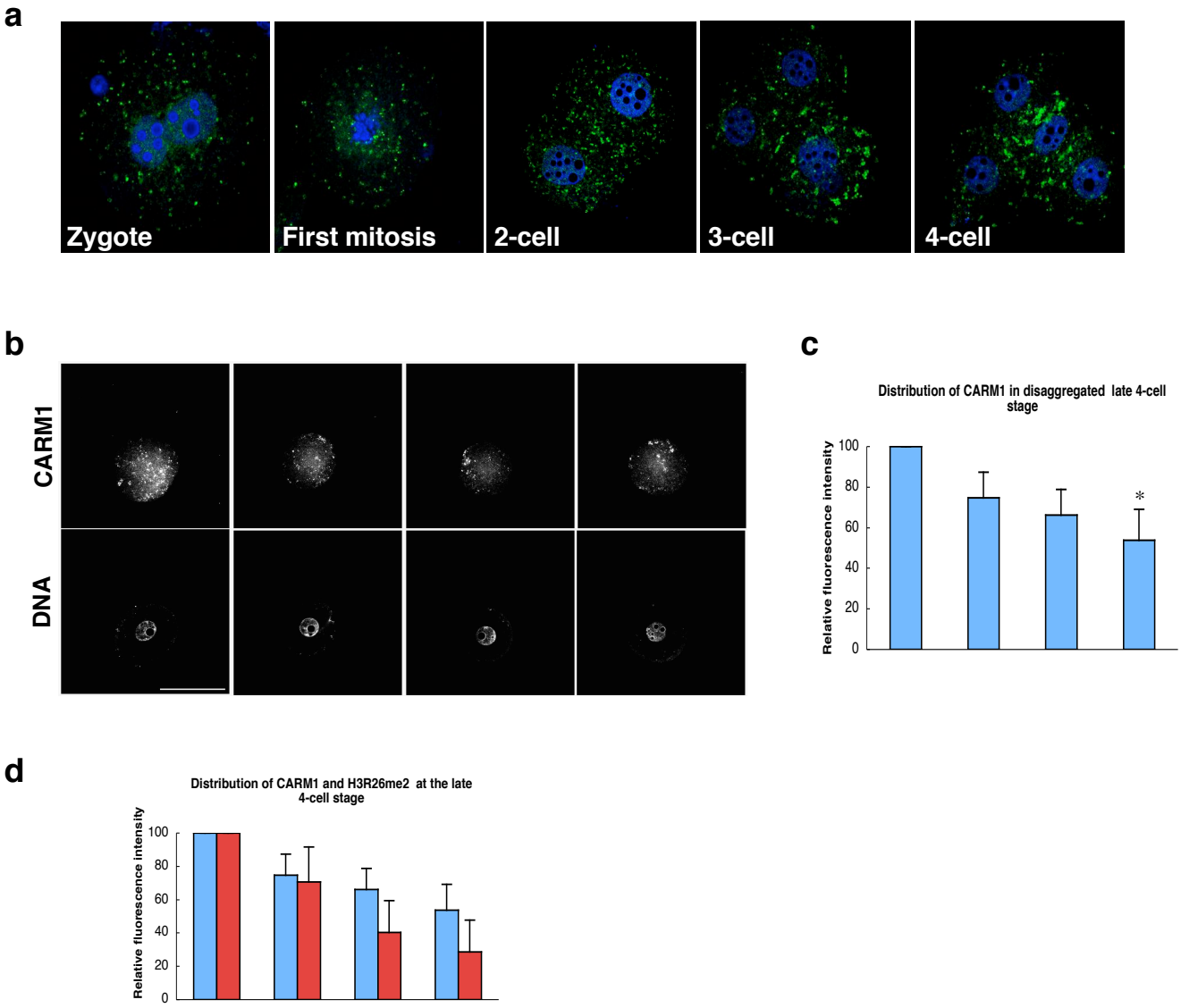

Figure S4. Localisation of CARM1 in the mouse preimplantation embryo: differential distribution of CARM1 in 4-cell stage blastomeres.

(a)CARM1 exhibits both nuclear and cytoplasmic localisation in dots throughout all the stages analysed. Embryos were collected following hormonal induction at the indicated stages, fixed and processed for immunostaining with a CARM1 antibody (Upstate, green). DNA is shown in blue. Images were captured using a 60x oil objective in a BioRad Radiance Upright Confocal Laser Microscope. Shown are representative single optical sections of 10 embryos examined per stage.

(b) Levels of CARM1 are different in the blastomeres of the 4-cell stage embryo

Freshly collected tetrahedral four cell stage embryos were processed for immunostaining with a CARM1 antibody. The cells of individual embryos were then disaggregated and scanned under confocal microscopy separately. Shown are projections of the 4 blastomeres of a representative embryo (n=8) . Sections were taken every 0.8 μm. Scale bar 10 μm.

(c)Total levels (nuclear and cytoplasmic) of CARM1 fluorescence were quantified through Z-planes using the Volocity software and normalised against the blastomere showing the highest level which was set at 100%. Decreasing values of fluorescence were calculated, normalised in each embryo and averaged accordingly (* p=0.0002). Each bar represents the relative fluorescence level of each of the 4-cell stage blastomeres.

(d) Comparison of the distribution of CARM1 (blue bars) and H3R26me2 (red bars) in tetrahedral (EM and ME) 4-cell stage embryos.

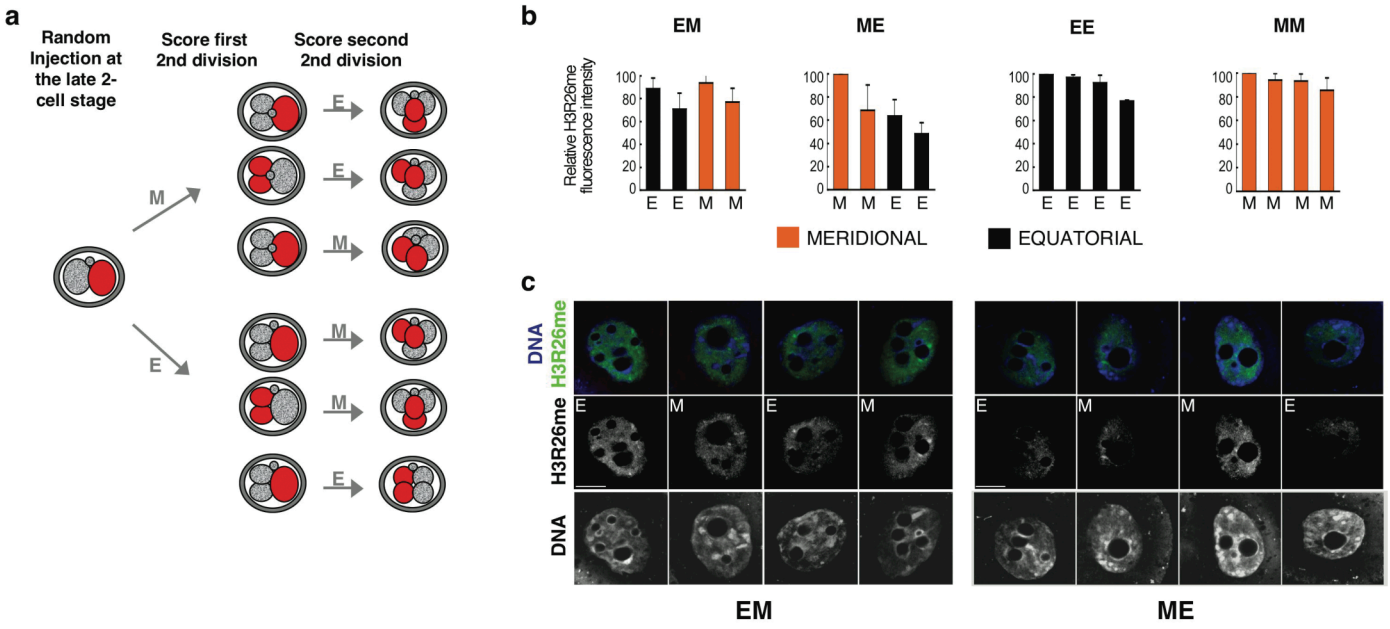

Figure S5. Levels of H3R26me correlate with the plane and order of division from the 2-cell stage

(a) Experimental design to group the embryos according to their division plane and order. A 2-cell stage blastomere was microinjected with rhodamine-coupled dextran and the embryos were monitored to determine the plane and order of division. Embryos were stained for H3R26me at the late 4-cell stage and levels of H3R26me were measured as in Figure 1.

(b) The sisters derived from late Equatorial divisions (ME embryos) display lower H3R26me levels than those derived from early Equatorial divisions (EM embryos) or from Meridional divisions. Embryos were grouped as EM (early division Equatorial, second division Meridional; n=23), ME (early division Meridional and second division Equatorial; n=20), EE (two Equatorial divisions; n=2) or MM (two Meridional divisions; n=3). H3R26me levels were analysed according to the presence of rhodamine-dextran, which allowed identification of the sisters derived from Equatorial (E cells, black bars) or Meridional (M cells, orange bars) divisions. In each embryo, levels of H3R26me were normalised to the highest blastomere, which was set at 100%. (Note that the MM and EE embryos are rare and constitute only ~20% of the embryos).

(c) Single optical sections of representative nuclei of one EM and one ME embryo . H3R26me shown in green,DNA in blue. Green and blue channels shown as grayscale at the bottom. Scale bar 10 μm. E or M cells are indicated.

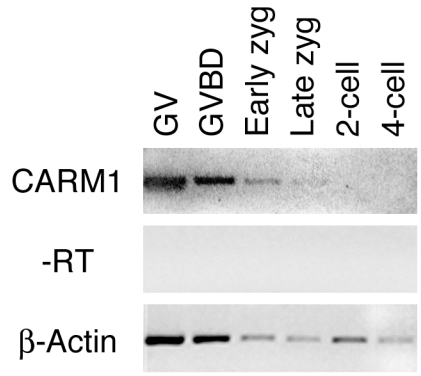

Figure S6.
CARM1 is expressed maternally and its transcripts are downregulated by the 2-cell stage. RT-PCR analysis of CARM1 was performed in freshly collected oocytes (15 oocytes), zygotes (15 embryos), 2-cell stage (15), and 4-cell stage embryos (5). cDNA samples were amplified for 35 cycles. *GV*, germinal vesicle, *GVBD*, germinal vesicle breakdown.

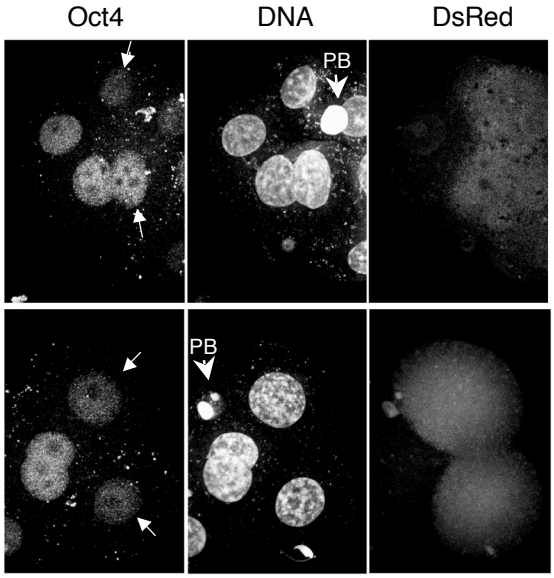

Figure S7.
Expression levels of Oct4 upon overexpression of CARM1 remain unchanged.
One blastomere of 2-cell stage embryos was injected with mRNA for CARM1.HA and DsRed. Embryos were analysed by immunofluorescence with an Oct4 antibody between the 6- and 8-cell stage. Shown are 4 nuclei of two different embryos, with two of them deriving from the CARM1 overexpressing blastomere (white arrows, note the presence of DsRed). Oct4 protein levels are variable and there was no correlation in this variation whether the blastomeres derived from the 2-cell stage blastomere injected with CARM1 mRNA or not (n=18) PB, polar body.

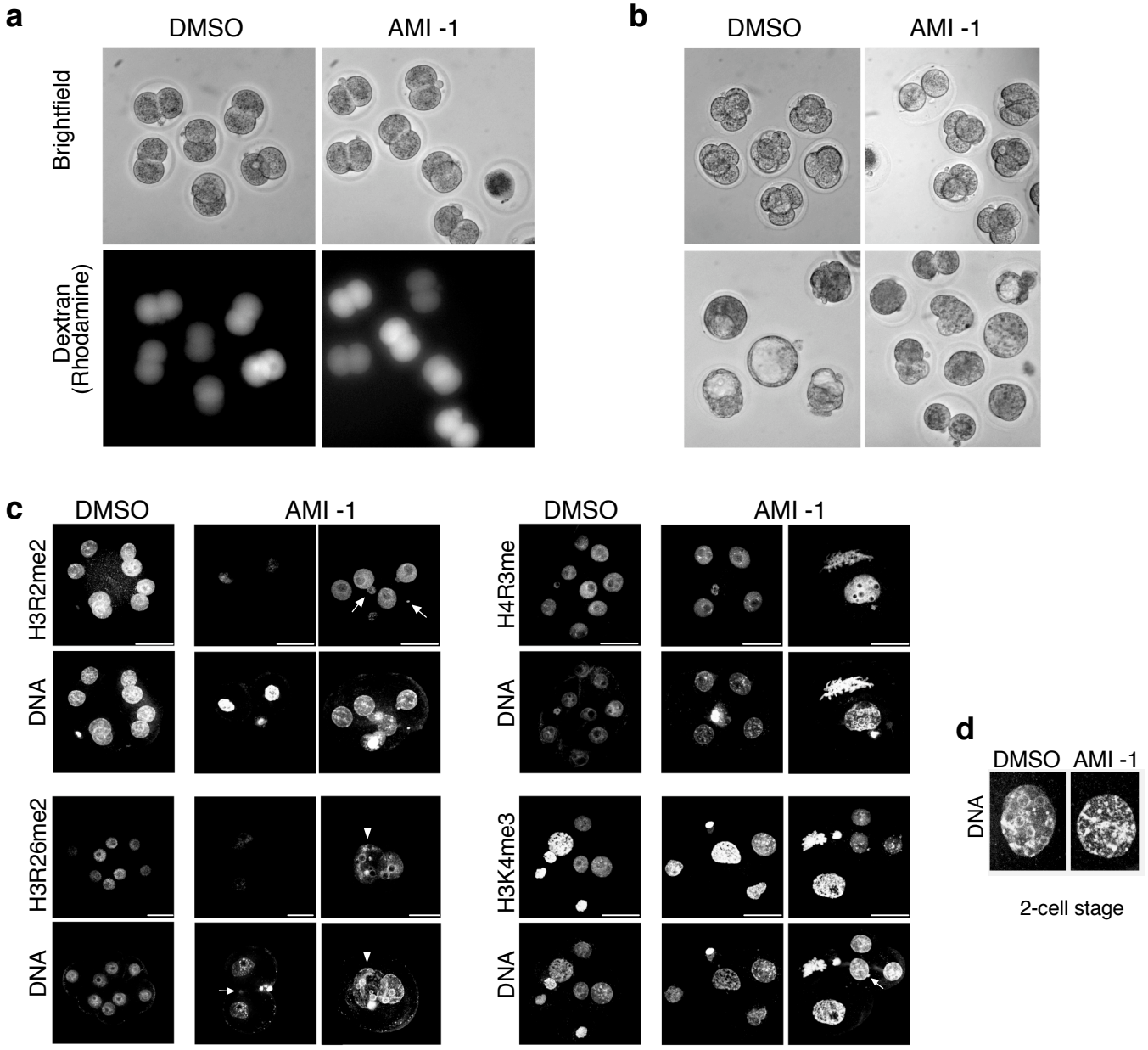

Figure S8.
Inhibition of arginine methyltransferase activity results in altered chromatin structure and impaired embryonic development.
Zygotes were microinjected with 100 μm AMI-1 or the vehicle (DMSO), together with Rhodamine-coupled Dextran as marker for injection. Note that AMI-1 shows some degree of cell permeability, albeit reduced (M. Bedford, pers.comm.). AMI-1 is a specific inhibitor of arginine methylation. In vitro, it inhibits CARM1 and to a lesser extent, PRMT1 (Cheng, D et al, 2004). In vivo, treatment of AMI-1 results in reduced levels of transcriptional activation that can be overcome by increasing amounts of PRMT1 and CARM1.
(a) Most embryos injected with AMI-1 proceeded to the 2-cell stage normally (71% n=43), although 19% either stopped their development or died at the zygote stage. All of the embryos injected only with DMSO reached to the 2-cell stage.
(b) AMI-1 treatment results in impaired development. Only 30% of AMI-1 injected embryos developped to the blastocyst stage, most of them arrested after the 1st or the 2nd cleavage (37% at the 2-cell stage, 8% at the 3- to 4-cell stage and 6% at the 8-16-cell stage). In contrast, most control (DMSO) embryos reached the blastocyst stage (80% n=38). Representative embryos after 3 and 4 days of culture are shown.

Figure S8. Continued.

(c) Reduced levels of histone arginine methylation upon AMI-1 treatment. Control and AMI-1 treated embryos were processed for immunostaining with the indicated antibodies. Most affected residues upon AMI-1 treatment were R2 and R26 of histone H3, which were largely reduced. Levels of H4R3me were not drastically affected and levels of H3K4me3 appeared unchanged. Note the differences in DNA staining between control and AMI-1 treated embryos and the unusual larger size of the pronucleus in the zygote (white arrowheads). AMI-1 treatment led to lagging chromatin in 26% of the embryos (white arrows). Both control (DMSO) and AMI-1-treated embryos were fixed at the same time. Samples for each antibody were processed in parallel. Shown are projections of representative embryos (1 for DMSO, 2 for AMI-1) of at least 6 embryos analysed per antibody and per grouop. Sections were taken every 1 μm. Scale bar is 30 μm.
(d) Inhibition of arginine methyltransferase activity results in altered nuclear organisation in the embryos. DNA staining of one nucleus of representative control (DMSO) and AMI-1 treated 2-cell stage embryos.

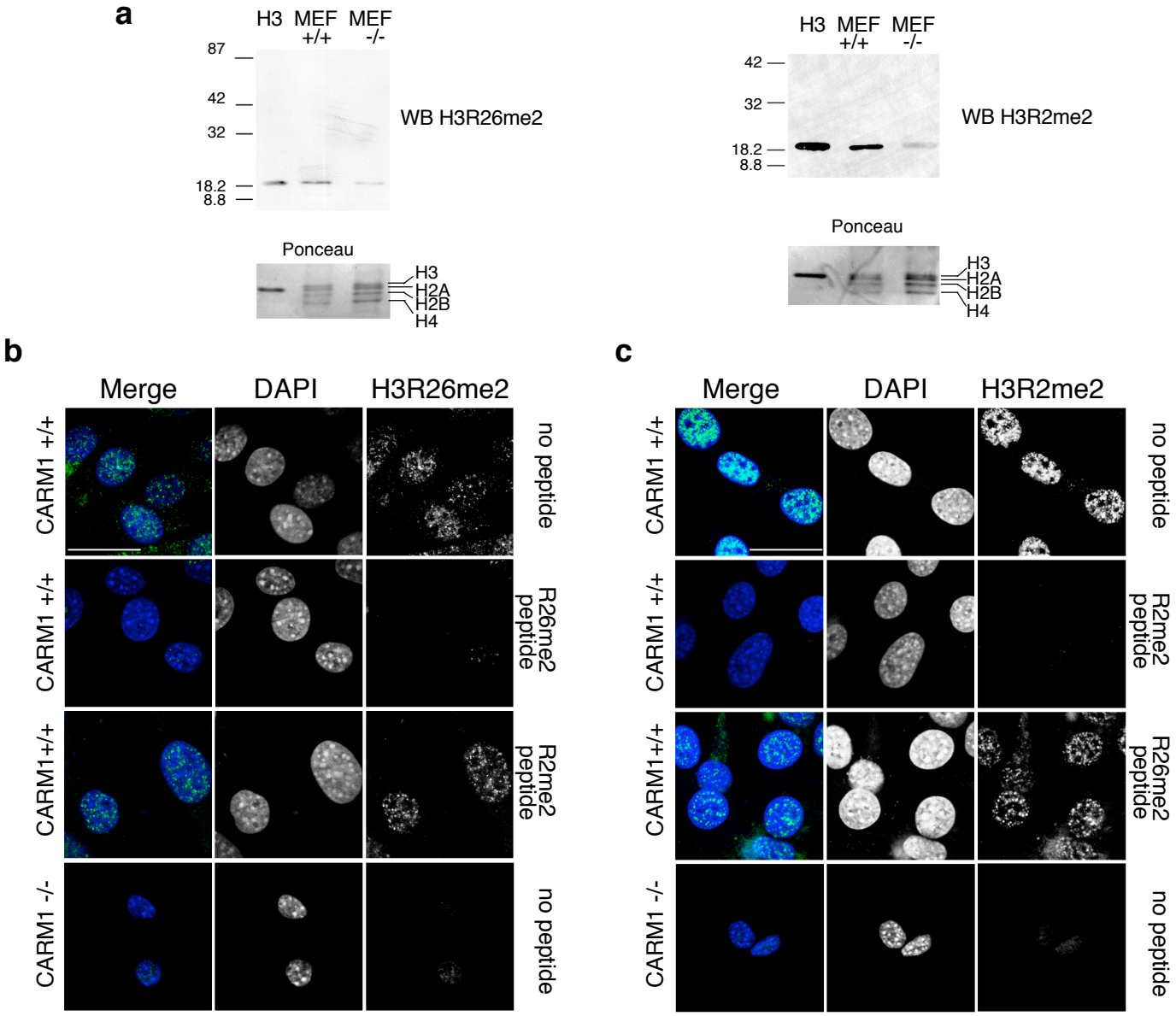

Figure S9.
Characterisation of the H3R26me2 and H3R2me2 antibodies.
(a)Total cell extracts of Mouse Embryonic Fibroblasts from CARM1+/+ or CARM1-/- homozygous mice or 500 ng of purified histone H3 (H3) were processed for Western Blot and probed with the indicated antibodies. Molecular weight markers (kDa) are indicated.
(b-c) Mouse Embryonic Fibroblasts derived from homozygous CARM1-/- or CARM1+/+ mice (Yadav, N et al. 2003) were processed for immunostaining with the H3R26me2 (b) or the H3R2me2 (c) antibodies. Distribution of both H3R26me2 and H3R2me is euchromatic. To confirm the specificity of these antibodies, we performed peptide competition by incubating the antibodies in the absence or presence of specific peptides as indicated. Note that the signal detected by the two antibodies is nuclear and that the H3R26me2 staining is lost when challenged with an H3 peptide dimethylated in R26 but unchanged when challenged with a peptide dimethylated in R2. Similarly, incubation of the H3R2me2 antibody with the dimethylated R2 peptide, but not with the dimethylated R26 peptide, abolishes staining. Accordingly, histone H3 methylation of R2 and R26 are reduced in the CARM1-/- cells. All panels are shown at the same magnification. Scale bar is 30 μm.

Table S1. Analysis of the distribution of the progeny of CARM1-overexpressing blastomere at the blastocyst stage

Embryo	No of Cells	No of Red Cells (% of all cells)	No of Inner (% of all cells)	Inner positive (% of inner)	% of red that are inner	No of Outer (% of all cells)	Outer Positive (% of Outer)	% of red that are outer
Carm1	23	9 (39)	7 (30)	7 (100)	77,78	16 (70)	2 (12.5)	22,22
Carm2	24	10 (42)	9 (38)	9 (100)	90,00	15 (62)	1 (6.6)	10,00
Carm3	21	8 (38)	9 (43)	6 (67)	75,00	12 (57)	2 (16.6)	25,00
Carm4	25	15 (60)	15 (60)	14 (93)	93,33	10 (40)	1 (10)	6,67
Carm5	29	14 (48)	11 (38)	10 (91)	71,43	18 (62)	4 (22)	28,57
Carm6	24	9 (38)	9 (38)	9 (100)	100,00	15 (62)	0 (0)	0,00
Carm7	34	8 (24)	11 (32)	7 (64)	87,50	23 (68)	1 (4.3)	12,50
Carm8	31	13 (42)	11 (35)	9 (82)	69,23	20 (65)	4 (20)	30,77
Carm9	31	14 (45)	10 (32)	10 (100)	71,43	21 (68)	4 (19)	28,57
Carm10	33	12 (36)	9 (27)	8 (89)	66,67	24 (73)	4 (16.6)	33,33
MEAN*	27.5	(41.17)	(37.36)	(88.53)	80.24	(62.64)	(12.76)	19.76
SD*	4.62	(9.32)	(9.11)	(13.73)	11.56	(9.250)	(7.35)	11.56

*Mean and SD in parenthesis correspond to the mean and SD of data expressed as percentage

Blastocysts derived from CARM1 overexpression experiments were fixed and stained with Phalloidin-Texas red to visualise the cell membrane. Confocal z-stacks were taken at 1μm intervals through the whole embryo. Imaris software was used to outline the cell membranes and from those create 3D models of all cells of the embryo. Cells were then scored according to their relative position: those cells completely surrounded by others are denoted as inner cells, those that have an outer surface denoted as outer cells. In conjunction, cells were scored as either positive or negative according to whether they expressed DsRed/CARM1 or not.

Key to table headings:

•No of Cells, is the total cell number in the blastocyst

•No of red cells, is expressed as absolute numbers and also relative (parenthesis) to the total number of cells in the blastocyst

•No of inner cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst

•Inner positive cells, expressed as number of positive cells that are inner and relative (parenthesis) to the total number of inner cells

•% of red cells that are inner, is expressed as the relative number of inner cells that are positive for CARM1/DsRed

•No of outer cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst

•Outer positive cells, are expressed as absolute number of positive cells that are outer and also relative (parenthesis) to the total number of outer cells

•% of red cells, that are outer is expressed as the relative number of outer cells that are positive for CARM1/DsRed

Table S2. Analysis of the distribution of the progeny of DsRed only -overexpressing blastomere (Control) at the blastocyst stage

Embryo	No of Cells	No of Red Cells (% of all cells)	No of Inner (% of all cells)	Inner positive (% of inner)	% of red that are inner	No of Outer (% of all cells)	Outer Positive (% of Outer)	% of red that are outer
DsRed1	26	13 (50)	6 (23)	4 (66)	30,77	20 (77)	9 (45)	69,23
DsRed2	28	14 (50)	10 (36)	7 (70)	50	18 (64)	7 (39)	50
DsRed3	26	11 (42)	6 (23)	4 (66)	36	20 (77)	7 (35)	64
DsRed4	32	12 (38)	9 (28)	7 (78)	58	23 (72)	5 (22)	42
DsRed5	36	16 (44)	10 (28)	6 (60)	38	26 (72)	10 (38)	62
DsRed6	32	14 (44)	7 (22)	6 (85)	43	25 (78)	8 (32)	57
DsRed7	29	13 (45)	7 (24)	5 (71)	38	22 (76)	8 (36)	62
DsRed8	34	16 (47)	7 (21)	4 (57)	25	27 (79)	12 (44)	75
DsRed9	29	13 (45)	11 (38)	7 (64)	54	18 (62)	6 (33)	46
DsRed10	31	15 (48)	12 (39)	6 (50)	40	19 (61)	9 (47)	60
MEAN	30.3	(45.31)	(28.1)	(66.9)	41.3	(71.9)	(37.1)	58.7
SD	3.3	(3.8)	(6.90)	(10.22)	10.26	(6.90)	(7.37)	10.26

*Mean and SD in parenthesis correspond to the mean and SD of data expressed as percentage

Blastocysts derived from DsRed only overexpression experiments were fixed and stained with Phalloidin-Texas red to visualise the cell membrane. Confocal z-stacks were taken at 1μm intervals through the whole embryo. Imaris software was used to outline the cell membranes and from those create 3D models of all cells of the embryo. Cells were then scored according to their relative position: those cells completely surrounded by others are denoted as inner cells, those that have an outer surface denoted as outer cells. In conjunction, cells were scored as either positive or negative according to whether they expressed DsRed or not.

Key to table headings:

•No of Cells, is the total cell number in the blastocyst
•No of red cells, is expressed as absolute numbers and also relative (parenthesis) to the total number of cells in the blastocyst
•No of inner cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst
•Inner positive cells, expressed as number of positive cells that are inner and relative (parenthesis) to the total number of inner cells
•% of red cells that are inner, is expressed as the relative number of inner cells that are positive for DsRed
•No of outer cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst
•Outer positive cells, are expressed as absolute number of positive cells that are outer and also relative (parenthesis) to the total number of outer cells
•% of red cells, that are outer is expressed as the relative number of outer cells that are positive for DsRed

Table S3. Analysis of the distribution of the progeny of CARM1(E267Q)-overexpressing blastomere at the blastocyst stage

Embryo	No of Cells	No of Red Cells (% of all cells)	No of Inner (% of all cells)	Inner positive (% of inner)	% of red that are inner	No of Outer (% of all cells)	Outer Positive (% of Outer)	% of red that are outer
E267Q1	36	18 (50)	13 (36)	9 (69)	50,00	23 (64)	9 (39)	50,00
E267Q2	37	19 (51)	13 (35)	7 (54)	36,84	24 (65)	12 (50)	63,16
E267Q3	30	16 (53)	11 (37)	9 (82)	56,25	19 (63)	7 (37)	43,75
E267Q4	30	16 (53)	11 (37)	6 (55)	37,5	19 (63)	10 (53)	62,5
E267Q5	30	14 (47)	11 (37)	6 (55)	42,86	19 (63)	8 (42)	57,14
E267Q6	34	13 (38)	11 (32)	6 (55)	46,15	23 (68)	7 (30)	53,85
E267Q7	31	14 (45)	10 (32)	5 (50)	35,71	21 (68)	9 (43)	64,29
E267Q8	35	19 (54)	12 (34)	7 (58)	36,84	23 (66)	12 (52)	63,16
E267Q9	32	13 (41)	13 (41)	5 (38)	38,46	19 (59)	8 (42)	61,54
E267Q10	33	15 (45)	12 (36)	5 (42)	33,3	21 (64)	10 (48)	66,7
MEAN*	32,8	(47.84)	(35.71)	(54.86)	41.39	(64.29)	(43.59)	58.61
SD*	2,62	(5.55)	(2.42)	(12.86)	7.32	(2.42)	(7.11)	7.32

*Mean and SD in parenthesis correspond to the mean and SD of data expressed as percentage

Blastocysts derived from CARM1(E267Q) overexpression experiments were fixed and stained with Phalloidin-Texas red to visualise the cell membrane. Confocal z-stacks were taken at 1μm intervals through the whole embryo. Imaris software was used to outline the cell membranes and from those create 3D models of all cells of the embryo. Cells were then scored according to their relative position: those cells completely surrounded by others are denoted as inner cells, those that have an outer surface denoted as outer cells. In conjunction, cells were scored as either positive or negative according to whether they expressed DsRed/CARM1(E267Q) or not.

Key to table headings:

•No of Cells, is the total cell number in the blastocyst
•No of red cells, is expressed as absolute numbers and also relative (parenthesis) to the total number of cells in the blastocyst
•No of inner cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst
•Inner positive cells, expressed as number of positive cells that are inner and relative (parenthesis) to the total number of inner cells
•% of red cells that are inner, is expressed as the relative number of inner cells that are positive for CARM1/DsRed
•No of outer cells, is expressed as absolute and relative (parenthesis) to the total number of cells in the blastocyst
•Outer positive cells, are expressed as absolute number of positive cells that are outer and also relative (parenthesis) to the total number of outer cells
•% of red cells, that are outer is expressed as the relative number of outer cells that are positive for CARM1/DsRed